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The Light Factor in Seed Health Testing

by

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The light factor in seed health testing

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Summary

Daily exposures of rice seeds to Sunlamp in the blotter test for 5, 10, 15, 20 and 30 minutes for seven days induced a level of infection of *Drechslera oryzae*, *Pyricularia oryzae* and *Trichoconis padwickii* lower than that obtained under standard light conditions. By exposing the seeds daily for 12 hours without removing the lids of the petri dishes, high counts of *T. padwickii* were obtained and counts of *D. oryzae* and *P. oryzae* increased considerably. Comparable or higher counts of the three fungi were obtained under the standard illumination by two Black Light tubes; one Black Light tube proved practically equally effective. Maximum counts of *Alternaria porri* f. sp. *dauci*, *Alternaria raphani*, *Drechslera avenae* and *P. oryzae* could still be obtained by initiating irradiation of seeds by Black Light after three days of incubation, whereas *D. oryzae*, *D. sorghicola*, *D. teres* and *T. padwickii* required daily exposures throughout the whole period of incubation. In routine seed health testing for these fungi it is, however, practical to use the same light regime, i.e. 12 hours daily exposure for seven days which is the accepted international standard.

Résumé

Le facteur lumière dans l'analyse sanitaire des semences

Des expositions quotidiennes de semences de riz, sous des lampes lumière du jour, en méthode papier buvard, d'une durée de 5, 10, 15, 20 et 30 minutes pendant sept jours, favorisent des taux d'infection par *Drechslera oryzae*, *Pyricularia oryzae* et *Trichoconis padwickii* inférieurs á ceux obtenus en condition de lumière standard. En exposant les semences tous les jours pendant 12 heures, sans retirer les couvercles des boites de Petri, des proportions élevées de *T. padwickii* furent obtenues, et les quantités de *D. oryzae* et de *P. oryzae* augmentèrent considérablement. Des comptages indentiques au supérieurs pour les trois champignons furent obtenus avec un éclairage standard de deux tubes fluorescents. Un seul tube fluorescent s'est montre également efficace. Des comptages maxima d'*Alternaria porri* f. *sp. dauci*, *Alternaria raphani*, *Drechslera avenae* et de *P. oryzae* feurent également être obtenus en provoquant l'irradiation des semences á l'aide de tubes fluorescents après trois d'incubation, alors que *D. oryzae*, *D. sorghicola*, *D. teres* et *T. padwickii* nécessitent des expositions journalières pendant toute la période d'incubation. Au cours des analyses sanitaires de semences de routine, four ces champignons, il est cependant pratique d'employer le même régime de lumière, par exemple: 12 heures par jour pendant sept jours est la norme internationale qui a été retenue.

Zusammenfassung

Licht als Faktor bei der Saatgutgesundheitsprüfung

Tägliche Belichtungen von Reissamen von 5, 10, 15, 20 und 30 minuten für 7 Tage mit der Jupiterlampe im Fliesspapiertest verursachten einen geringeren Infektionsumfang bei *Drechslera oryzae*,

Pyricularia oryzae und *Trichoconis padwickii* als man unter Standard-Lichtbedingungen erhielt. Durch tägliche 12-stündige Belichtung der Samen ohne die Deckel der Petrischale zu entfernen, erzielte man hohe Auszählungen von *T. padwickii* und die Zahlen von *D. oryzae* und *P. oryzae* nahmen beträchtlich zu. Vergleichbare oder höhere Zahlen der 3 Pilze erhielt man unter der Standardbeleuchtung durch zwei Leuchtstoffröhren, eine Leuchtstoffröhre erwies sich als praktisch gleich wirksam. Höchstzahlen von *Alternaria porri* f. sp. *dauci*, *Alternaria raphani*, *Drechslera avenae* und *P. oryzae* konnten erreicht werden durch anfängliche Bestrahlung der Samen mit Leuchtstoffröhren nach dreitägiger Inkubation, während *D. oryzae*, *D. sorghicola*, *D. teres* und *T. padwickii* tägliche Belichtungen während der gesamten Inkubationsperiode benötigten. Bei der Routine-Gesundheitsprüfung auf diese Pilze ist es jedoch praktisch, die gleiche Lichtform zu benützen, d. h. 7 Tage lang eine 12-stündige Belichtung, was den angenommenen internationalen Standard darstellt.

Introduction

Light, temperature and humidity are known to influence profoundly the reproduction of fungi. General growth characteristics, extent of sporulation, spore morphology and pigmentation, which are markedly influenced by light, are of special interest for detection and identification of fungi in routine seed health testing. Leach (1967) pointed out the advantage of illuminating seeds under test and proposed a standard method for using light in routine testing to the Committee on Plant Diseases of the International Seed Testing Association (Neergaard, 1965). For this procedure a daily 12 hours exposure of seeds to the fluorescent Black Light tubes (320–420 nm) during the incubation period was recommended, and has since then been generally adopted.

The fluorescent Black Light tubes emit radiation within the range of near ultraviolet to visible light (320–420 nm). It gives a much higher proportion of near ultraviolet at 360 nm which appears to be most suitable for routine seed health testing. This wavelength is effective in inducing sporulation without adverse effects even at high dosages (Leach, 1967), and a high proportion of the radiation is transmitted through many plastics and certain types of glass, particularly Pyrex (Howell, 1964; Leach, 1967; Koller, 1965). This makes irradiation practicable because the lids of petri dishes can be kept on during incubation and loss of moisture from the blotters is prevented.

There are many sources of light which emit near ultraviolet radiation, such as fluorescent daylight tubes, sunlamps and germicidal lamps (Koller, 1965). The quality and intensity of these sources differ. Leach (1967) reported that the most effective wavelength inducing sporulation is 290 nm, which is present in higher proportion in the Sunlamp. He suggested that irradiation of seeds with Sunlamp for only a short period may be enough to be effective. There are, however, some obvious disadvantages in using short wavelengths. These are strongly absorbed by most plastics and glass containers and the lids of petri dishes should therefore be removed during irradiation; moreover, excessive dosages inhibit sporulation.

The fluorescent Sunlamp used in this investigation is a polychromatic lamp which does not only emit short wavelengths below 300 nm but also a considerable proportion of the effective photoinductive wavelengths. Sunlamp was compared with Black Light as used in routine seed health testing of rice (*Oryza sativa* L.) for *Drechslera oryzae*

(van Breda de Haan) Subram., *Pyricularia oryzae* Cav. and *Trichoconis padwickii* Ganguly.

The influence of the number of daily 12 hour light exposures during incubation on infection percentages of seed-borne fungi in rice and a few other crops was studied.

Materials and methods

The standard blotter method for the detection of seed-borne fungi, recommended by the International Seed Testing Association, was used as control in all the experiments, with 12 hours of irradiation each day for seven days with two Black Light tubes. As normally used, two 40 W fluorescent Black Light tubes emitting spectrum from 320 to 420 nm were placed in a horizontal position 20 cm apart at a height of approximately 41 cm above the seed. The intensity of the two tubes (Philips TL 40 W/08) on the plane area 60 × 110 cm where the seeds were placed measured 187–230 $\mu W/cm^2$ in the near ultraviolet region.

One fluorescent Sunlamp tube (Westinghouse FS 40) was used; this emits a spectrum ranging from 265–405 nm. It was placed 41 cm above the seeds, thus covering an area of approximately 60 × 110 cm. The intensity of light near the seeds was 44–66 $\mu W/cm^2$. The tube emits a large amount of invisible ultraviolet (far and near) energy in the range of 280–350 nm. Very little visible light is emitted and is of the soft blue type.

The intensity of light from the two Black Light tubes is more than double that produced by one Sunlamp tube. Hence, in one of the experiments, the effect of only one Black Light tube, hung at the distance of 41 cm above the seed, on infection percentages was investigated; the light intensity measured was 90–100 $\mu W/cm^2$.

Fifteen rice seed samples received from Egypt, India, Indonesia, and South Korea were included in the experiments. They were infected with *D. oryzae*, *P. oryzae*, and *T. padwickii*.

In general, 400 untreated seeds of a sample per treatment, plated on moist blotters contained in plastic petri dishes, were examined for fungi seven days after incubation at 20 °C (± 1 °C).

In the first few experiments under Sunlamp, the seeds, both uncovered and covered by the lids of the petri dishes, were irradiated for different lengths of time. In the case of Black Light tubes, the lids were never removed.

The sporulation and infection percentages of *P. oryzae* were also studied in complete darkness. The effect of different exposures of Sunlamp on the growth and sporulation of rice pathogens was also studied in pure culture.

In an experiment on the influence of different numbers of daily exposures six treatments were set up. In the first, infected seeds were kept in complete darkness for one day followed by irradiation of 12 hours each day for six days. In the 2nd, 3rd, 4th, 5th and 6th treatments, infected seeds were irradiated for 12 hours each day after keeping them in complete darkness for 2, 3, 4, 5, and 6 days, respectively, (figure 1). The infection counts were made after seven days. These treatments, in addition to the

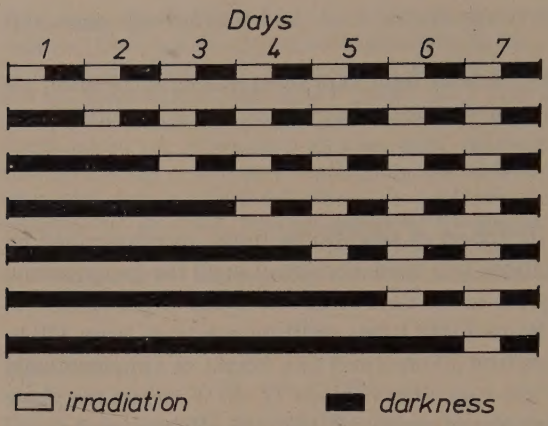

Fig. 1. Schematic presentation of the experimental procedure of irradiation.

treatment under the standard light condition as control (12 hours NUV irradiation each day for seven days), were applied to five rice seed samples infected with *D. oryzae*, *P. oryzae* and *T. padwickii*. Four hundred seeds per treatment of each sample were used.

Treatments having four and five exposures were selected and compared with the standard light condition. A completely randomised block design was used with 400 seeds for each treatment, 25 seeds per plate. Records of infection counts of fungi in one plate were regarded as one unit observation. Each experiment was repeated twice. This design was applied to another set of five rice seed samples infected with the three rice pathogens. Five other crops, barley (*Hordeum vulgare* L.), carrot (*Daucus carota* L.), oats (*Avena sativa* L.), radish (*Raphanus sativus* L.) and sorghum (*Sorghum bicolor* (L.) Moench) infected with *Drechslera teres* (Sacc.) Shoemaker, *Alternaria porri* (Ell.) Neerg. f. sp. *dauci* (Kuhn) Neerg., *Drechslera avenae* (Eidam) Scharif, *Alternaria raphani* Groves & Skolko and *Drechslera sorghicola* (Lef. & Sherw.) Richardson & Fraser, respectively, were also selected for this experiment.

Results

Growth of fungi in culture under Sunlamp

In complete darkness, only few conidia were seen in the colonies of *P. oryzae* while *D. oryzae* and *T. padwickii* remained sterile. Cultures of the three pathogens sporulated well when they were irradiated with a single exposure of Sunlamp for 15 minutes after three days of incubation but with increased exposures exceeding 30 minutes the growth of *P. oryzae* was retarded; less retardation was noticed in the other two fungi.

Since sporulation of *P. oryzae* was not materially increased by light, rice seed sample no. 1 was plated on blotters and incubated in complete darkness. The infection counts were made after different incubation periods and the results are presented in table 1.

Table 1. Percentages of infection for *Pyricularia oryzae* in sample no. 1 under darkness, Sunlamp and the standard light condition (100 seeds per treatment).

Day(s) of incubation in darkness	Percentage infection in darkness	Irradiated with single exposure on	Percentage infection, counted after 7 days		
			Sunlamp		Standard light condition (2 Black Light tubes 12 hr daily for 7 days)
			1 min	5 min	
1	16	1st day	24	22	
2	24	2nd „	30	32	
3	26	3rd „	22	34	
4	26	4th „	22	26	
5	16	5th „	24	32	
6	18	6th „	22	26	
7	8	7th „	24	28	32*

* pooled from 7 experiments.

After 24 hours of incubation the fungus could only be recognised at the embryo end of the infected seed. Many of the short silvery grey conidiophores were devoid of conidia while others bore only one or few conidia. The total infection counts increased considerably within two to three days of incubation whereas they were considerably lower when recordings were made after four, five and six days. This indicated that the fungus can effectively be counted already after two to three days of incubation in complete darkness. Such an observation was also made by Kang, Neergaard and Mathur (1972) when rice seeds were incubated in light.

Irradiation of seeds by Sunlamp in the blotter test

a. *Lids removed during irradiation.*

Effect of single exposures. To ensure whether Sunlamp influences the sporulation and infection counts of *P. oryzae*, a small experiment was carried out with rice sample no. 1 known to be heavily infected with *P. oryzae*.

Single exposures of Sunlamp for one and five minutes were applied after different incubation periods in complete darkness, and the counts were made after seven days. For this experiment, lids of petri dishes were removed during irradiation in order to avoid the absorption of the short wavelengths by plastic lids.

Irradiation of seeds for a single exposure of five minutes after incubation in complete darkness generally gave slightly higher percentages of infection than one minute exposure (table 1). The counts obtained with five minutes exposure were almost the same as obtained by the standard light condition (12 hours NUV daily exposure for seven days).

Effect of different lengths of exposures. Daily exposures of 5, 10, 15, 20, 30 minutes and 1, 2, 4, 8 and 12 hours were applied on a rice sample infected with the three important rice pathogens. The lids of the petri dishes were removed during irradiation

Table 2. Percentage infection of fungi in rice sample no. 2 under standard light condition, darkness and Sunlamp. Records made 7 days after incubation (400 seeds per treatment).

Treatment	Percentage infection		
	P. oryzae	*D. oryzae*	*T. padwickii*
Standard light condition (2 Black Light tubes)	8.8	11.5	37.5
Darkness	0.5	1.5	0
Sunlamp 5 min each day	7.8	8.5	3.8
10 „ „ „	7.8	9.3	1.8
15 „ „ „	8.0	9.0	0
20 „ „ „	6.5	7.5	1
30 „ „ „	6.8	10.3	0
1 hr „ „	4.5	9.0	0
2 „ „ „	2.0	8.3	0
4 „ „ „	3.5	7.3	0
8 „ „ „	3.3	5.3	0.3
12 „ „ „	4.3	4.5	0.3

and the irradiated plates were kept in complete darkness before and after the light exposure. The results are tabulated in table 2.

Exposure for short periods of five to 30 minutes each day yielded rather satisfactory counts of *P. oryzae* and *D. oryzae* whereas only low counts of *T. padwickii* were obtained. Higher doses of one to 12 hours reduced infection percentages of *Pyricularia* and *Drechslera* considerably. Counts of these two fungi in complete darkness were almost negligible and *Trichoconis* did not develop at all. Growth of the latter fungus was almost inactivated under Sunlamp.

When lids were removed for longer periods, such as for one to 12 hours, loss of moisture invariably occurred in proportion to the period of exposure, and water was added to keep the blotters wet.

Table 3. Comparison of percentages of infection for three fungi in rice sample no. 2, irradiated three times daily for different lengths of exposures, with standard light condition and complete darkness (400 seeds per treatment).

Treatment	Percentage infection		
	P. oryzae	*D. oryzae*	*T. padwickii*
Standard light condition (2 Black Light tubes)	8.8	11.5	37.5
Darkness	0.5	1.5	0
Sunlamp three times 5 min each day	8.0	8.3	16.8
10 „ „ „	7.8	8.5	11.8
15 „ „ „	8.8	10.5	11.3
20 „ „ „	7.8	11.0	8.3
30 „ „ „	4.8	7.5	6.8

Irradiation repeated three times each day

An attempt was made to improve the infection counts by increasing the frequency of exposure of Sunlamp. Therefore, the seeds were irradiated three times a day throughout the incubation period, the lengths of exposures being 5, 10, 15, 20 and 30 minutes.

Repeated radiation did not increase the infection counts of *P. oryzae* and *D. oryzae* compared with those obtained by single exposure (tables 2 and 3). However, infection counts of *T. padwickii* increased considerably when exposures of five to 15 minutes were repeated three times each day, but the recorded percentages of 11.3 to 16.8 were still very low compared with 37.5 obtained under the standard light condition.

b. *Lids not removed during irradiation.*

Effect of daily 12 hours exposure. Removal of lids during irradiation had three disadvantages. Firstly, it is impractical in routine work, and secondly there is loss of moisture from the blotters making conditions different from the standard light conditions. Although water was added after irradiation, the moisture content in the dishes fluctuated. The third disadvantage is that Sunlamp emits small amounts of far ultraviolet radiation which quickly inhibits sporulation. Therefore, in this experiment the lids of the petri dishes were not removed. In routine work this would cut down the labour involved in removing and replacing the lids, avoid loss of moisture and still allow to pass approximately 80% of the light with wavelength of 310 nm (Leach, 1967). This radiation may be enough for the stimulation of sporulation.

Since infection percentages of *T. padwickii* were only affected by 12 hours Sunlamp exposure, daily exposure for this period was tried on two rice samples, no. 1 and 2 (table 4).

Exposure to Sunlamp gave infection percentages of *P. oryzae* and *D. oryzae* comparable to those obtained in the standard light condition. *T. padwickii* still appeared in much less counts under Sunlamp.

It appears from these results that it is not necessary to take off the lids during irradiation of Sunlamp and hence lids were not removed in all the subsequent experiments.

Table 4. Percentages of infection for the three fungi using daily 12 hours exposure of Sunlamp versus the standard light condition for 7 days (400 seeds per treatment).

Treatment	Percentage infection		
	P. oryzae	*D. oryzae*	*T. padwickii*
Sample no. 1			
Standard light condition (2 Black Light tubes)	32.1	5.2	27.1
Darkness	14.5	0.5	0
Sunlamp	28.8	6.5	14.8
Sample no. 2			
Standard light condition (2 Black Light tubes)	7.9	12.6	25.9
Darkness	2.8	0.5	0
Sunlamp	10.3	12.0	20.5

Irradiation for different lengths of time by Sunlamp and Black Light tubes

The main purpose of this investigation was to find out whether the infection counts from the two Black Light tubes are still higher than the counts from Sunlamp when seed samples are irradiated for the same length of time. The results are presented in table 5.

With slight variation recorded, the incidence of *P. oryzae* and *D. oryzae* was more or less the same when exposed to Black Light tubes and Sunlamp for a given period of time. There was an increase in the infection counts with increase in exposure time. In most cases, the highest counts were made at 12 hours exposure daily. Because of profuse sporulation, it was also easy to recognise the growth of fungi in this treatment and, in general, incidence was more with two Black Light tubes.

Table 5. Effect of similar exposure from two tubes of Black Light (2 BL) and one tube of Sunlamp (SL) on rice sample no. 1 and 3 (400 seeds per treatment).

Daily Exposure Time	Accumulated number of infected seeds					
	P. oryzae		*D. oryzae*		*T. padwickii*	
	2 BL	SL	2 BL	SL	2 BL	SL
Sample no. 1						
1 min	41	32	0	2	0	0
5 „	48	45	0	0	0	0
10 „	48	43	1	2	0	0
20 „	52	70	1	5	2	3
30 „	60	88	6	2	0	0
1 hr	82	74	4	4	2	1
2 „	107	93	7	5	4	1
4 „	109	93	4	9	2	3
8 „	102	78	22	10	16	5
12 „	130	101	21	22	62	25
Sample no. 3						
1 min	5	4	0	11	0	1
5 „	4	3	0	14	2	1
10 „	12	6	13	18	7	12
20 „	7	2	15	12	4	23
30 „	8	5	13	21	4	5
1 hr	4	11	21	28	6	13
2 „	15	12	21	16	19	3
4 „	13	7	15	25	21	10
8 „	8	15	30	31	47	77
12 „	14	10	32	31	89	80

BL = Black Light tubes
SL = Sunlamp

Irradiation by Sunlamp after different initial incubation periods in darkness versus the standard light condition

The previous experiment showed that 12 hours daily light exposure gave reliable results both with Black Light tubes and Sunlamp. The counts were less when the duration of exposure was short.

The effect of these two sources of light was also evaluated in another way. Daily 12 hour light exposure of Sunlamp and two Black Light tubes was applied after incubating seeds in darkness for 1, 2, 3, 4, 5 and 6 days, the lids of petri dishes not being removed. The infection counts were made after seven days of incubation (fig. 1).

The infection counts of the three fungi in all treatments under Sunlamp were lower than the counts from Black Light (table 6) including the standard light treatment and decreased with decreasing dosages (with the exception of *Pyricularia* under six exposures to Black Light).

Effect of different intensities from Sunlamp and Black Light tubes

The previous studies were carried out on the basis of dosages regardless of the intensities of light given out by these tubes. When the tubes are hung 41 cm from the seeds, one Black Light tube emits an intensity of 100 $\mu W/cm^2$, two tubes of Black Light give 211 $\mu W/cm^2$, whereas one tube of Sunlamp emits only 55 $\mu W/cm^2$. The results may not be conclusive because of these differences and the role of intensity therefore had to be considered. Fourteen rice samples were tested under the three mentioned conditions by exposures of 12 hours each day for seven days.

The percentages of infection of *P. oryzae* and *T. padwickii* were, generally, slightly higher under one and two Black Light tubes than under one tube of Sunlamp (tables 7 and 9). Since the difference under one and two tubes of Black Light was very small, the high and low intensities of Black Light tubes have practically the same effect on

Table 6. Infection percentages of the three fungi in sample no. 2 under Sunlamp and two Black Light tubes (400 seeds per treatment).

Daily 12 hr exposure of NUV	Percentage infection					
	P. oryzae		*D. oryzae*		*T. padwickii*	
	2 BL	SL	2 BL	SL	2 BL	SL
7 exposures	7.9*	10.3	12.6*	12.0	25.9*	20.5
6 ,,	11.5	7.5	11.3	8.5	22.0	15.0
5 ,,	6.8	7.5	11.5	7.3	21.8	14.3
4 ,,	7.8	4.5	11.3	8.5	26.3	17.3
3 ,,	8.0	6.8	10.0	8.3	21.3	14.8
2 ,,	9.5	4.5	8.5	6.0	14.0	12.0
1 ,,	4.3	3.3	6.5	5.5	6.5	6.3

BL = Black Light tubes
SL = Sunlamp
* Standard light conditions

Table 7. Accumulated infection counts for *Pyricularia oryzae* in 14 rice seed samples incubated under different intensities of Sunlamp and Black Light. Daily exposure for 12 hours.

Sample No.	Number of infected seeds			
	Darkness	One Sunlamp Intensity 55 $\mu W/cm^2$	One Black Light tube Intensity 100 $\mu W/cm^2$	Two Black Light tubes Intensity 211 $\mu W/cm^2$
1	41	95	112	130
2	4	32	35	40
3	9	7	12	12
4	10	34	79	67
5	3	3	19	8
6	4	3	7	5
7	0	0	1	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
Total infection in 14 × 400 seeds	71	172	265	262

these two fungi. The results obtained for *D. oryzae* showed that the highest infection counts were obtained with two tubes of Black Light and one tube of Sunlamp and the incidence was somewhat lower with one Black Light tube (table 8). This was exhibited profoundly in samples 8, 11, 12, 13 and 14. The reaction of this fungus was different from that of the two other fungi. The growth of *D. oryzae* observed by naked eye was more distinctly limited on seeds under Sunlamp (figure 2) while under Black Light it was more diffuse, spreading out on to the blotter. On the other hand, under the compound microscope the morphological characteristics of this fungus developed under the two sources of light did not show any recognisable difference.

Effect of one Sunlamp and one Black Light tube at the same intensity

The quality of these two tubes is different. The Black Light tube emits a high amount of near ultraviolet radiation at a wavelength of 360 nm while the peak emission of Sunlamp is approximately at 310 nm. By changing the hanging distance of one Black Light tube to 74 cm, the intensity was brought down to that produced by a Sunlamp, 55 $\mu W/cm^2$ at a height of 41 cm. The effect was studied on six rice samples (table 10).

The infection counts of *P. oryzae* under Sunlamp were comparatively lower than the counts under Black Light, whereas *D. oryzae* appeared slightly more from seeds irradiated by Sunlamp. The counts for *T. padwickii* were more or less the same under the two conditions.

Table 8. Accumulated infection counts for *Drechslera oryzae* in 14 rice seed samples incubated under different intensities of Sunlamp and Black Light. Daily exposure for 12 hours.

Sample No.	Number of infected seeds			
	Darkness	One Sunlamp Intensity 55 $\mu W/cm^2$	One Black Light tube Intensity 100 $\mu W/cm^2$	Two Black Light tubes Intensity 211 $\mu W/cm^2$
1	1	22	16	21
2	1	26	27	49
3	0	43	28	29
4	0	7	6	7
5	0	23	15	28
6	0	6	2	7
7	1	65	80	68
8	7	322	159	277
9	2	110	107	122
10	0	101	81	85
11	8	234	136	250
12	5	190	128	227
13	6	350	258	347
14	8	268	153	260
Total infection in 14 × 400 seeds	39	1767	1221	1777

Table 9. Accumulated infection counts for *Trichoconis padwickii* in 14 rice seed samples incubated under different intensities of Sunlamp and Black Light. Daily exposure for 12 hours.

Sample No.	Number of infected seeds			
	Darkness	One Sunlamp Intensity 55 $\mu W/cm^2$	One Black Light tube Intensity 100 $\mu W/cm^2$	Two Black Light tubes Intensity 211 $\mu W/cm^2$
1	0	25	60	62
2	0	35	84	73
3	0	40	54	66
4	0	1	1	0
5	0	38	29	64
6	0	25	29	26
7	0	144	216	157
8	0	277	184	230
9	0	213	262	197
10	0	130	105	68
11	0	18	25	42
12	0	15	18	29
13	0	2	7	8
14	0	2	3	7
Total infection in 14 × 400 seeds	0	965	1077	1029

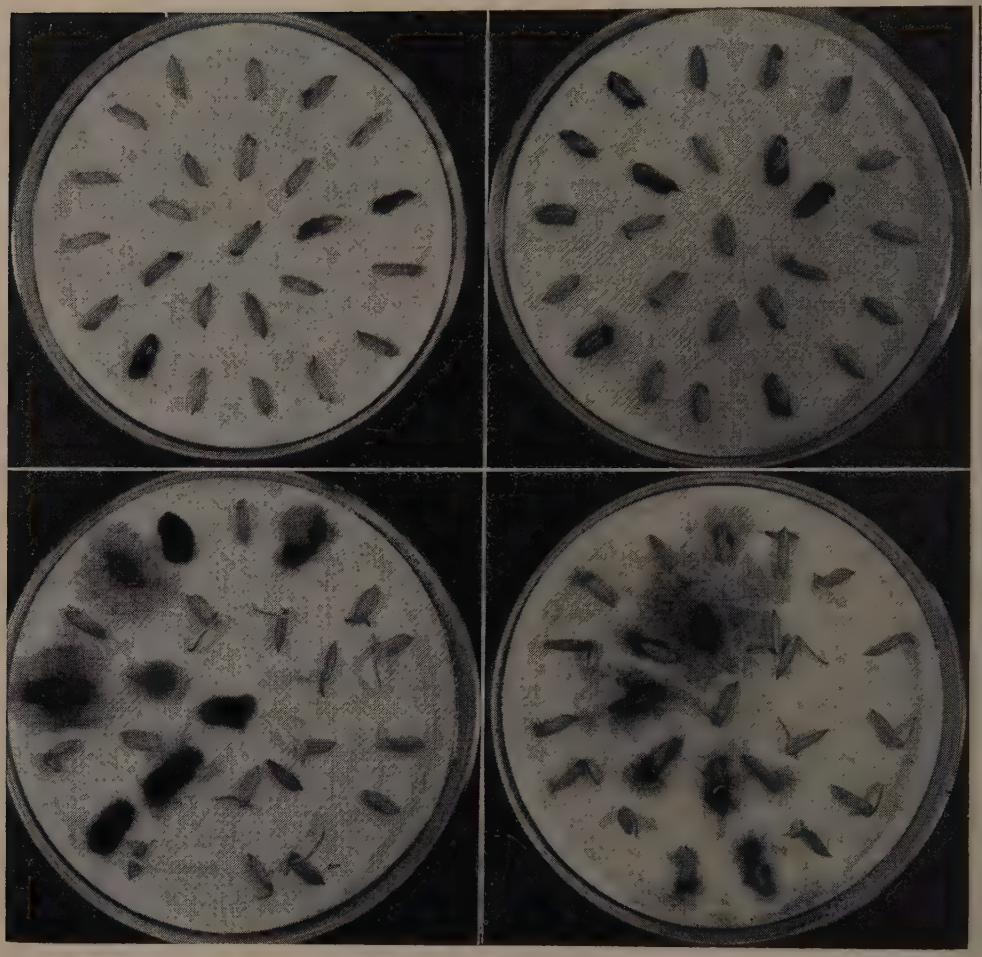

Fig. 2. Limited growth of *Drechslera oryzae* on infected seeds of samples 13 and 14 exposed to Sunlamp, 12 hours daily (top dishes). Profuse and spreading growth in the two samples under 12 hours daily exposure of Black Light (lower dishes).

Exposing seeds for increasing numbers of 12 hour periods with two Black Light tubes

The results of the preliminary experiment are tabulated in table 11. Exposing seeds three to six times gave infection counts of *P. oryzae* and *T. padwickii* comparable to those obtained when the seeds were exposed seven times. Infection counts of *D. oryzae* were rather low when seeds were exposed 4, 5 or 6 times to NUV compared with seven light exposures.

In the second experiment the effect of four and five light exposures was tested against the standard of seven exposures and the results, with statistical interpretations, are included in table 12.

Table 10. Accumulated infection counts of three fungi under the two sources of light at an intensity of 55 μW/cm². Daily exposure for 12 hours.

Sample No.	Number of infected seeds					
	P. oryzae		*D. oryzae*		*T. padwickii*	
	BL	SL	BL	SL	BL	SL
1	146	97	24	18	96	59
2	46	30	33	27	87	102
3	0	0	59	84	119	137
7	0	0	134	196	228	243
11	0	0	163	218	28	36
15	22	9	22	29	120	86
Total infection in 6 × 400 seeds	214	136	435	572	678	663

BL = Black Light
SL = Sunlamp

Table 11. Total counts of *Pyricularia oryzae*, *Drechslera oryzae* and *Trichoconis padwickii* in five rice seed samples under different light exposures (400 seeds per treatment).

Daily 12 hr exposure of NUV	*Pyricularia oryzae* Seed sample no.					Total number of infected seeds in 5 × 400 seeds
	1	2	3	7	18	
7 exposures	123	32	0	0	1	156
6 „	118	46	0	1	0	165
5 „	126	27	0	1	0	154
4 „	119	31	0	0	1	151
3 „	129	32	0	0	0	161
2 „	99	38	0	2	0	137
1 „	76	17	0	1	0	94
Daily 12 hr exposure of NUV	*Drechslera oryzae* Seed sample no.					Total number of infected seeds in 5 × 400 seeds
	1	2	3	7	18	
7 exposures	26	91	91	82	100	390
6 „	25	45	77	66	104	317
5 „	22	46	85	66	106	325
4 „	23	45	85	52	100	305
3 „	16	40	82	53	99	290
2 „	13	34	62	50	82	241
1 „	18	26	73	43	77	237
Daily 12 hr exposure of NUV	*Trichoconis padwickii* Seed sample no.					Total number of infected seeds in 5 × 400 seeds
	1	2	3	7	18	
7 exposures	55	104	105	214	41	519
6 „	67	88	121	184	27	487
5 „	65	87	139	196	43	530
4 „	48	105	116	186	23	478
3 „	52	85	88	161	12	398
2 „	19	56	67	104	23	268
1 „	18	26	43	34	6	127

Table 12. Average counts of infection of *Pyricularia oryzae*, *Drechslera oryzae* and *Trichoconis padwickii* from three experiments under different light exposures.
Bold print: Differences not statistically significant. (400 seeds per treatment).

	Average counts of infection for *Pyricularia oryzae*						F	LSR	
	Number exposures		Number exposures		Number exposures			2 means	3 means
Sample no. 1	7	112.3	**5**	**119.3**	**4**	**130.3**	3.07	14.56	15.34
„ 2	**5**	**28.7**	**4**	**34.7**	**7**	**36.7**	1.56		
	Average counts of infection for *Drechslera oryzae*						F	LSR	
	Number exposures		Number exposures		Number exposures			2 means	3 means
Sample no. 1	4	15.3	**5**	**24.7**	**7**	**25.3**	4.74	7.28	7.67
„ 2	**4**	**30.7**	**5**	**39.3**	**7**	**41.0**	2.36		
„ 7	4	55.0	**5**	**69.0**	**7**	**76.7**	6.11	12.32	12.98
„ 16	**4**	**146.0**	**7**	**159.0**	**5**	**161.3**	2.76		
„ 17	**4**	**132.0**	**5**	**140.7**	**7**	**145.3**	1.32		
	Average counts of infection for *Trichoconis padwickii*						F	LSR	
	Number exposures		Number exposures		Number exposures			2 means	3 means
Sample no. 1	4	46.3	7	58.0	5	76.3	12.79	11.76	12.39
„ 2	4	59.3	7	73.0	5	90.0	9.82	13.72	14.46
„ 7	4	201.7	**7**	**224.3**	**5**	**232.0**	9.68	14.28	15.05
„ 16	**5**	**148.3**	**4**	**155.3**	7	172.7	5.34	14.84	15.64
„ 17	4	108.3	5	134.7	7	173.0	35.89	17.64	18.59

Samples 7, 16, and 17 were not tested for *Pyricularia oryzae* as they were infected only in trace amounts (table 11).

Pyricularia oryzae. There was little difference in the results obtained by the three treatments. In sample no. 2 the results were not statistically different between the three treatments, and in sample no. 1 they were not different between four and five exposures.

Drechslera oryzae. No statistical difference was found between the results from five and seven exposures, whereas four exposures yielded somewhat lower counts, statistically different from the two other treatments in only two of the five samples.

Trichoconis padwickii. The highest counts were obtained in the treatments of five and seven exposures in four of the five samples, being statistically significant.

Other fungi (*table 13*). Development and infection counts of *A. porri* f. sp. *dauci*, *A. raphani* and *D. avenae* were not affected significantly by variation of light exposures. Seven exposures yielded significantly higher counts of *D. sorghicola* and *D. teres* than did four and five exposures.

Table 13. Average counts of infection of *Alternaria porri*, *A. raphani*, *Drechslera avenae*, *D. sorghicola* and *D. teres* from three experiments on the effect of three light exposures.
Bold print: Differences not statistically significant. (400 seeds per treatment).

Fungi	Average counts of infection						F	LSR	
	Number exposures		Number exposures		Number exposures			2 means	3 means
A. porri f. sp. *dauci*	**7**	**46.3**	**5**	**48.3**	**4**	**50.3**	1		
A. raphani	**7**	**86.7**	**4**	**88.0**	**5**	**90.3**	1		
D. avenae	**4**	**236.0**	**7**	**237.7**	**5**	**241.0**	1		
D. sorghicola	**4**	**69.3**	**5**	**75.3**	7	96.3	10.36	12.32	12.98
D. teres	4	72.7	5	85.7	7	112.7	22.19	12.04	12.69

Discussion

Cultural studies indicated that *D. oryzae* and *T. padwickii* require light for sporulation while *P. oryzae* showed sparse sporulation in complete darkness and was enhanced by light exposure. This behaviour of *P. oryzae* in culture has also been observed by Leach (1962a) and Chakrabarti and Wilcoxson (1970). Kang, Neergaard and Mathur (1972) established that all three fungi sporulated well and recorded infection percentages were high when seeds were exposed to near ultraviolet radiation (NUV) supplied by Black Light tubes.

The Sunlamp emits more effective photo-inductive wavelengths (290 nm and 310 nm) than the Black Light tubes but it also produces inhibitory wavelengths. Leach (1968) reported heavy reduction of conidial formation in *Stemphylium botryosum* Wallr. at 280 nm when cultures were exposed for a few seconds only; this wavelength is present in Sunlamp. But irradiation of rice seeds with exposures of 5 to 30 minutes of Sunlamp after removal of the lids of the dishes was enough to induce sporulation and to obtain infection counts of *D. oryzae* and *P. oryzae* comparable with those obtained by the standard procedure using two Black Light tubes. On the contrary, these treatments had adverse effects on *T. padwickii* (table 2). The influence was the same even when the time of exposure was increased from few minutes to 1 to 12 hours. Under these circumstances also the infection percentages of the other two fungi were adversely affected. The reduction may have been due to the prolonged exposures to inhibitory wavelengths which may have inactivated the growth and caused inhibition in the conidial formation as observed by Leach (1967, 1968) for other fungi.

Removal of the lids of petri dishes during long exposures results in excessive loss of moisture from the blotters, and seeds were therefore irradiated with short daily exposures. By this the infection counts of *T. padwickii* could be increased considerably but were still less than those obtained by using the standard light exposure (NUV 12 hours each day). This may indicate that the fungus requires intermittent short

exposures of Sunlamp and needs high energy of radiation, but repeated irradiation and taking off of lids each time is highly impractical.

Effect of shorter and longer duration of Sunlamp irradiations without removing the lids was therefore tried. In doing so, the short wavelengths are cut out, only wavelengths longer than 310 nm being able to pass through. Short periods of daily exposures induced sporulation in the three fungi. Leach (1961) reported that exposure of 30 seconds of near ultraviolet radiation was enough to induce sporulation in *D. oryzae* and the fungus fruited profoundly when long exposures were given. Kang, Neergaard and Mathur (1972) reported similar effects on rice fungi, the infection percentages increasing with increasing dosages of NUV radiation. In the present investigation the highest counts were obtained when the seeds were exposed for 12 hours each day to Sunlamp or Black Light.

Comparison of the effect of different intensities of light showed that the infection counts of *P. oryzae* and *T. padwickii* were practically the same under one tube as under two tubes of Black Light. Leach (1967) stated that one or two Black Light tubes are satisfactory for inducing sporulation in many seed-borne fungi. The counts of *D. oryzae* were, however, in the present investigation generally low under one tube emitting intensity of 100 $\mu W/cm^2$ (table 8). *D. oryzae* may need the higher energy of radiation provided by two tubes (211 $\mu W/cm^2$). When the same amount of energy of radiation was given to the three fungi, infection counts of *P. oryzae* under Black Light tubes were higher than under Sunlamp, counts of *D. oryzae* were slightly higher under Sunlamp while those of *T. padwickii* were almost the same under the two light sources. It thus appears that it is not only the quality of light which matters, the organisms to be irradiated should be considered before drawing conclusions, and even different isolates of the same fungus may vary in this respect. Leach (1962b) found that when 40 isolates of *Ascochyta pisi* Lib. from vetch seeds were grown in darkness, sporulation ranged from none to quite profuse. Chakrabarti and Wilcoxson (1970) reported that light was not necessary to induce sporulation in *P. oryzae* while it was concluded by Oh, Cho and Lee (1965) that light was essential for sporulation, a contradiction that may be due to differences between isolates. This is clearly illustrated by Honda and Sukamoto (1968) for *D. oryzae* where one isolate sporulated in darkness only after near ultraviolet irradiation while another isolate sporulated freely in complete darkness. Two types of growth were observed in *T. padwickii* by Mathur, Mallya and Neergaard (1972). One produced more mycelium and few conidia while the second fruited profoundly having less mycelium. These two types of growth, which were also observed in the present investigation, belong perhaps to two different biotypes which behave differently under the same set of light condition. According to Leach (1967) these phenomena are the innate factors which may be responsible for conflicting results.

Leach (1967) suggested that it may not be necessary to start NUV irradiation of plated seeds until mycelium begins to grow out. Such a trend was observed in our investigations on *A. porri* f. sp. *dauci*, *A. raphani*, *D. avenae* and *P. oryzae* where seeds could be incubated in complete darkness for three days before being exposed to light.

A different trend was observed in *D. oryzae*, *D. sorghicola*, *D. teres* and *T. padwickii* which needed daily exposure throughout the incubation period of seven days in order to obtain high infection counts. Leach (l.c.) also reported that one NUV exposure is sufficient to induce sporulation but that daily exposures cause more extensive sporulation with increasing increment of growth thereby making recognition and identification easier. Muskett (1938) incubated oat seeds for three days in complete darkness before he exposed them to Sunlamp for 20 minutes. This statement induced sprorulation in *D. avenae*. De Tempe (1957) followed this procedure, but used a germicidal lamp as a source of irradiation. He exposed oat seeds to it for five minutes at a distance of 14 cm, and obtained high counts of *D. avenae*. On the contrary, Howell (1964) irradiated oat seeds with Black Light tubes continuously for seven days after one day of initial incubation in complete darkness, and obtained a five times higher count of *D. avenae* than when the seed was kept in complete darkness for eight days. Calpouzos and Lapis (1970) studied the effect of the duration of light exposure on formation of pycnidia and conidia of *Septoria nodorum* Berk. by using cool, white fluorescent tubes and found that with longer exposures of light, up to 14 days, the fungus produced larger numbers of pycnidia, and the percentages of fertile pycnidia increased from 0 to 95%. Moreover, he found that light exposure at an earlier or later stage of incubation resulted in fewer or no pycnidia.

It is inferred from our results as well as those of many other workers that sporulation and detection of fungi depend not only on the type and duration of light but also on the species and biotype.

The tested fungi can be divided into two groups. The first group, *D. oryzae*, *D. sorghicola*, *D. teres*, and *T. padwickii*, requires high frequency of irradiation for induction of sporulation relevant to seed health testing, while the second group, consisting of *A. porri* f. sp. *dauci*, *A. raphani*, *D. avenae*, and *P. oryzae*, is less responsive to light in this regard.

The practice of using as a standard procedure two Black Light tubes at a defined distance from the seed with daily exposure of 12 hours for seven days is substantiated.

Conclusions

1. *D. oryzae* and *T. padwickii* need light for sporulation while *P. oryzae* can sporulate in complete darkness, but submission to light increases sporulation.
2. Sunlamp (one) can be employed in recording *D. oryzae*, *P. oryzae* and *T. padwickii* effectively in seed health testing of rice. Seeds should be exposed 12 hours each day for seven days without taking off the lids. This treatment equals two Black Light tubes currently used in routine seed health testing in many laboratories.
3. Further work should be done to explore the usage of Sunlamp in irradiating seeds infected with fungi other than those reported in this paper.
4. Seed health testing for the three rice fungi can also be performed effectively using only one Black Light tube instead of two. Further studies are necessary to draw conclusions relevant to testing seeds of other crops.

5. Seed health testing of rice for *P. oryzae* needs only four exposures of NUV of 12 hours duration while *D. oryzae* and *T. padwickii* need six to seven exposures for recording maximum infection. To provide uniform conditions, a practical compromise is seven exposures.
6. *A. porri* f. sp. *dauci*, *A. raphani* and *D. avenae* are less sensitive to NUV than *D. sorghicola* and *D. teres*.

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